EXHIBIT B

RT-PCR ASSAY PROTOCOLS

Preparation of total RNA from tumor samples

RNA isolation and purification using RNeasy was carried out following the manufacturer's protocol:

Samples of tissue of normal mammary gland, tumor-free tissue of breast carcinoma biopsies and ductal invasive breast cancer biopsies were collected/provided by the Pathology Department at the Göttingen University Medical School (Dr. Bernhard Hemmerlein).

Ten to thirty milligrams of tissue was homogenized in Buffer RLT in a microcentrifuge tube. The homogenate was centrifuged for 3 minutes at maximum speed and the supernatant was transferred to a new microcentrifuge tube. An equal volume of 70% ethanol was added to the cleared lysate and gently mixed. The RNA-containing lysate was applied to the RNeasy column and the column was centrifuged to remove the flow through. Buffer RW1 was applied to the column, the column was centrifuged for 15 seconds at >8000 x *g*. The supernatant flow through discarded. The column was washed twice with RPE buffer and centrifuged for 2 minutes >8000 x *g* to dry the column. The RNA was eluted from the column with water. The RNA was then converted to double-stranded cDNA using SuperScript II reverse transcriptase.

First strand synthesis

Total RNA (25–50 μg) was mixed with 100 pmol oligo(dT) plus 900nM EAG specific primer (5'-CGGAGCAGCCGGACAA) and heated to 70 C for 10 minutes and then quick chilled on ice. First-strand reaction buffer (50 mM Tris-HCl at pH 8.3, 75 mM KCl, 3

mM $MgCl_2$), 10 mM DTT and 10.5 mM dNTP mixture was added to the RNA–oligo mixture, mixed gently and heated at 45°C for 2 minutes. SuperScript II reverse transcriptase (200 units) was added to the reaction mixture, which was incubated at 45°C for 1 hour. The reaction was terminated by placing the mixture on ice.

Second strand synthesis

The second strand synthesis was carried out with the product of the first-strand reaction, in the presence of 20 mM Tris-HCl, pH 6.9, 90 mM KCl, 4.6 mM $MgCl_2$, 150 nM β–NAD^+, 10 mM $(NH_4)_2SO_4$, 200 nM dNTP mixture, 10 units *E. coli* DNA ligase, 40 units *E. coli* DNA polymerase I and 2 units *E. coli* RNase H. The reaction mixture was mixed gently and incubated at 16°C for 2 hour, followed by the addition of 20 units of T4 DNA polymerase and further incubation at 16°C for 5 minutes. The synthesis reaction was terminated by the addition of 15 mM EDTA. The cDNA was extracted, precipitated and resuspended in water.

PCR reactions via TaqMan assay

Using the primers described above, PCR cycles were carried out as follows: The initial thermocycle consisted of 2 minutes at 50°C for annealing and/or extension, followed by 10 minutes at 95°C for denaturation. The subsequent amplification cycles consisted of 40 cycles of 15 seconds at 95°C for denaturation, 15 seconds at 56°C for annealing and 60 seconds at 60°C for extension.